# A NEW MODEL OF DOUBLE PIPET HOLDER AND THE TECHNIC FOR THE ISOLATION OF LIVING ORGANISMS *

F. HECKER
*Kansas City, Missouri*

The working parts of the old models of double pipet holders are made of stock brass. In the new model to be described here they are made of cast phosphor bronze. The male and the female milling of the old models are cut in the working parts; the male milling of the new model is cut separately and fastened to the working part with screws. In the old models after a short period of use, lost motion develops in the milling, destroying the accuracy of the instrument. Also, when the working part is moved upward, the male milling immediately passes out of its bearing; in the new model the male milling is never disengaged. A space of 5/1000 inch is left between the working part and the face of the milling, and in this space there are 5 brass shims, each of which is 1/1000 inch in thickness. Thus if the bearings after a time become loose, the trouble is quickly remedied by unscrewing the screws and removing the working part and one of the thin sheets of brass, then replacing the working part and gradually tightening the screws until the working part is easily operated with the millhead. In the older models, the bearings of the transverse carriages are too short, so that when they move outward from the center of the instrument, they develop a marked looseness. In the new model, this defect has been entirely overcome. An arm projects at right angles to the perpendicular carriage (Fig. 1); on this arm is a carriage traveling at right angles to the perpendicular carriage. On the upper surface of this carriage there is another carriage which travels at right angles to the one supporting it, and on the upper surface of this carriage an arm projects towards the center of the instrument. This is the pipet holder.

The carriage has the female milling cut on one of its surfaces, while on the opposite surface of this part there is a clamp which fits a small metal block extending from the side of the stage of the microscope. When the millhead 1d is tightened, a metal clamp is moved upward to engage the block projecting from the side of the stage of the microscope, thus making the instrument secure and rigid. The male milling 1B is fastened to a face plate, which engages the female milling, and to this face plate the perpendicular carriages are made fast with screws; the transverse carriage is operated by the

* Received for publication February 25, 1916.

millhead 1A, which when turned moves the transverse carriage backward and forward, and by this movement the perpendicular carriages are moved forward and backward. The advantage of the position of the millhead in this model, as compared with the older models, is that one does not have to reach around in front of the microscope to operate this carriage. At the opposite end of the screw operated by the millhead 1A, is a small knurled screw 1C. This screw when tightened, takes out all the lost motion in the screw which operates the carriage. Thus not only can the working parts be adjusted by the removal of the shims, but the screws likewise can be adjusted when they become loose

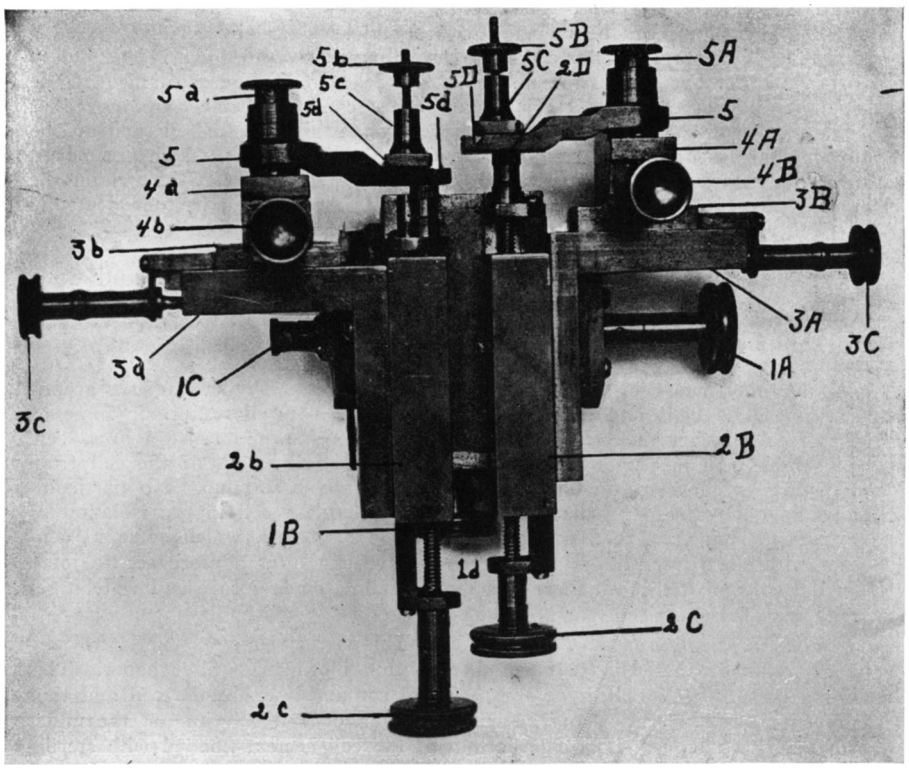


Fig. 1. The new model of double pipet holder.

in their bearings. The perpendicular carriages 2B, 2b are operated by the millheads 2C, 2c. These millheads when turned, cause the carriages to move up or down. At the opposite ends of the screws operated by the millheads 2C, 2c are small knurled thumb screws of the same type as 1C. These, when tightened, adjust the lost motion developed by the operating screws in their bearings. Extending at right angles to the perpendicular carriages is an arm which is part of each of the carriages 2B, 2b, and is marked 3A, 3a. On the upper surface of these arms are the carriages 3B, 3b, operated by the mill-

heads 3C, 3c. When the millheads 3C, 3c are turned, these carriages move at right angles to the perpendicular carriages. On the upper surfaces of the carriages 3B, 3b are the carriages 4A, 4a, operated by the millheads 4B, 4b and moving at right angles to 3B, 3b. On the upper surfaces of these carriages are the clamps for the pipets proper 5, 5, and the bearings of the clamps. If the thumb screws 5A, 5a are loosened, the clamp can be moved to any desired angle, and the thumb screws 5A, 5a again tightened to make the angle of the pipet secure. At the opposite end of the arm of the pipet clamp to the bearing are other thumb screws 5B, 5b, which, when operated, press on the plate clamps 5C, 5c fitting over the grooves 5D, 5d; by this operation the pipet is held securely in the grooves 5D, 5d.

The two pipets can be moved forward and backward by turning the millhead 1A. The perpendicular carriages can be moved up and down independently of each other by turning the millheads 2C, 2c. On the upper surface of the arm of the perpendicular carriages are other carriages, which are moved at right angles to the perpendicular carriages by the millheads 3C, 3c, and on the upper surfaces of these carriages is another set of carriages, which are moved at right angles to the carriages 3B, 3b by the millheads 4B, 4b, and on the upper surface of these carriages is the long arm which can be turned at any desired angle by loosening the thumb screws 5A, 5a.

Having described the parts of the double pipet holder and their operation, I shall now explain the accessory apparatus, and the preparation of it for the isolation of living bacteria.

*Isolating Cell.*—The cells used for the isolation of living bacteria are of 2 types, glass cells and brass cells. Dr. Barber (of whose technic some of the steps here are modifications) uses the glass cell, while I use a brass cell. This brass cell measures 80 mm. by 27 mm. and the height of the cell from the glass window to the upper edge of its walls is 15 mm. No particular advantage is offered by the brass cell over the glass cell in the isolation of organisms, but the broad upper edges of the brass cell which come in contact with the cover slip, when coated with vaselin offer a more secure joint. In the bottom of the cell there is a glass window made secure and water-tight with gum balsam.

*The Preparation of the Isolating Cell.*—This is very simple. The lateral and back walls of the cell are covered with thin blotting or filter paper. This lining acts as an equalizer of the moisture contained within the cell and thus prevents the evaporation of the culture media or salt solution on the under surface of the cover slip. The bottom of the cell is next flooded with sterile-distilled water. The upper edges of the cell which come in contact with the cover are then thoroughly coated with vaselin. The vaselin holds the cover slip in position and lutes the point of contact of the cover slip with the edges of the cell, making an air-tight joint between them.

To protect the living organisms from a possibly fatal change of temperature in the transfer from the diseased part to the under surface of the cover slip I have devised an electrically heated jacket which, when placed around the isolating cell, maintains its temperature at 35.5-37.5 C. A further advantage of this jacket is that one does not have to hurry in the isolation of living organisms for fear of their dying before the transplantation from the under surface of the cover to the test tube or to the animal can be accomplished.

*Preparation of the Cover Slip.*—The cover slip used is of 2 sizes, 50 by 24 mm. and 40 by 24 mm. The cover slip is washed with soap and running water, rinsed in running warm water, and thoroughly dried with a piece of clean muslin. It is then given a coating of vaselin. Excess of vaselin is thoroughly removed from the cover slip with another clean piece of muslin, and then the surface is polished with Japanese bibulus paper. The vaselin on the surface of the cover slip prevents the fine droplets of condensation water from uniting to form large drops. After the cover slip has been prepared,

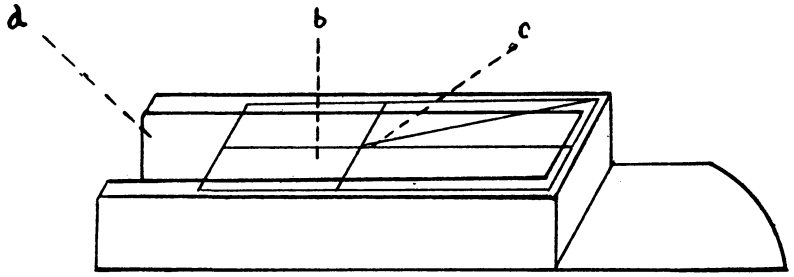


Fig. 2. (*a*) The isolating cell. (*b*) The ruled cover slip. (*c*) The ruled cover slip in position on the cell.

it is passed through a gas flame several times to insure sterilization. It is now placed on the cell.

Its upper surface is ruled as follows: A fine capillary pipet, drawn from a piece of glass tubing, is covered with thick India ink and the point placed at the outer edge of the cover slip. The instant that the pipet comes in contact with the cover slip the entire pipet is brought in contact with the surface of the cover slip. The point of the pipet is now drawn from the front of the

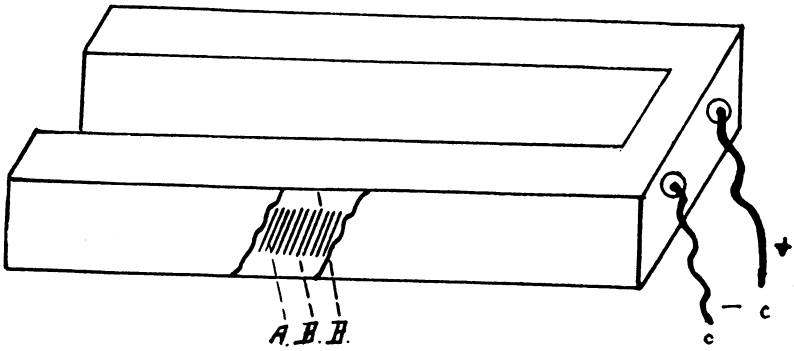


Fig. 3.—Electrically heated jacket for the isolating cell. A is the heating element, B, B is the asbestos insolation, and C, C the terminals of the heating element.

cell, backward; this movement leaves a black line on its surface. The pipet is again covered with India ink and another line made at right angles to the one passing through the longest diameter of the cover. A third line is drawn to make an angle of 95 degrees with each of the previous lines. The completed cover slip has 3 lines, none of which is parallel to another. The line

which is at 95 degrees to the other two lines, is used as a guide for the placing of the droplets containing the isolated bacteria.

*Placing the Droplets on the Under Surface of the Cover Slip.*—A pipet is drawn and the excess of the capillary portion over 10 to 15 cm. is broken off. From 10 to 15 mm. of the end of the pipet are bent at right angles to the pipet. A piece of rubber tubing is then attached to the opposite end. The free end of the rubber tubing is between the lips, and the point of the pipet is immersed in broth or sterile normal salt solution; by gentle suction the desired quantity of liquor is drawn into the pipet. Now the placing of a series of small droplets around a large central drop on the under surface of

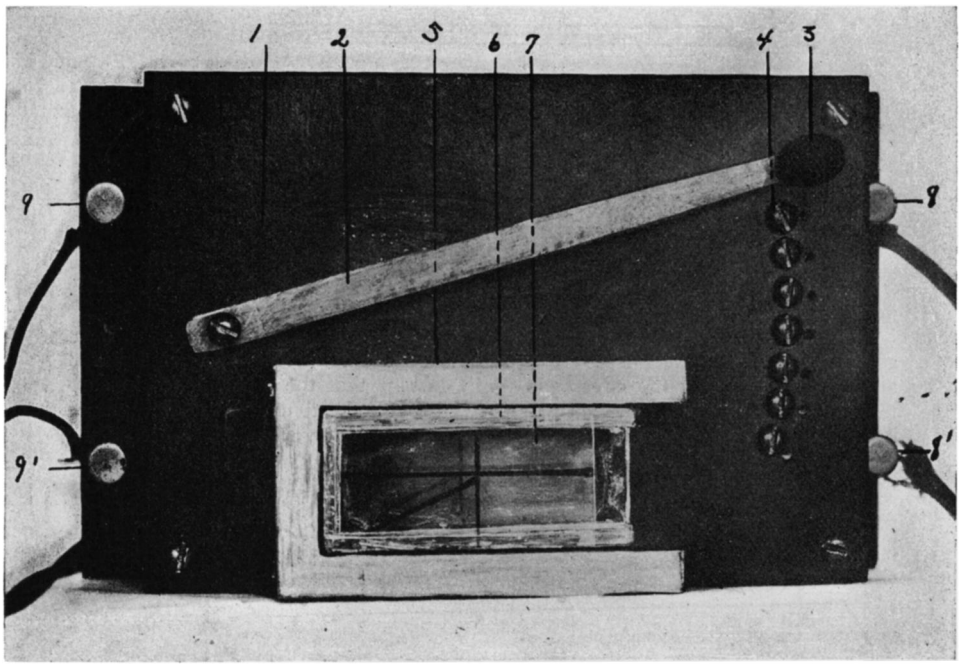


Fig. 4. Rheostat for controlling the temperature of the warm jacket and the cell. Beginning at 1, which is the rheostat proper, 2 indicates the arm of the rheostat, used for making the contact points; 3 is the handle of the lever; 4, the contact points which control the amount of electricity entering the coil in the warm jacket; 5, the warm jacket; 6, the isolating cell surrounded by the warm jacket; 7, the cover slip in position on the surface of the cell, and on its surface the ruling used; 8 and 8′ are the two poles of the incoming electricity; and 9 and 9′ are the outgoing poles, which are connected with the coil in the heating jacket. Any desired temperature can be obtained with this instrument around the cell.

the cover slip is accomplished with the point of the pipet. The object of the small droplets around the central drop is to insure the latter against rapid evaporation. A coarse capillary pipet is now made. It is charged with sterile distilled water, the point passed under the cover slip, and a series of small droplets made on the under surface of the cover slip at its outer edge. These droplets equalize the moisture in the cell and thus prevent the evaporation of

the droplets on the under surface of the cover which contain the emulsion of bacteria, and also the very small droplets which hold the isolated bacteria in suspension.

*The Inoculation of the Central Drop.*—A small sterile platinum loop is used for the collection of pus from the focus of suppuration, after which the loop is cautiously passed to the central drop, and its pus content thoroughly mixed into that drop. The mixing having been completed, the cell is placed under a bell glass jar or large Petri dish until the pipets have been made and charged.

*The Making of Capillary Pipets for Isolation Purposes.*—The apparatus consists of a pair of small thumb forceps and 2 Bunsen burners, one of which is remodeled in such a way as to offer a flame for making the very fine capillary pipets needed for the isolation of bacteria. The glass tubing used for making the capillary pipets has a caliber of 2 mm. and is cut into 14-cm. lengths. It is held by the thumb, index, and second fingers of the left hand

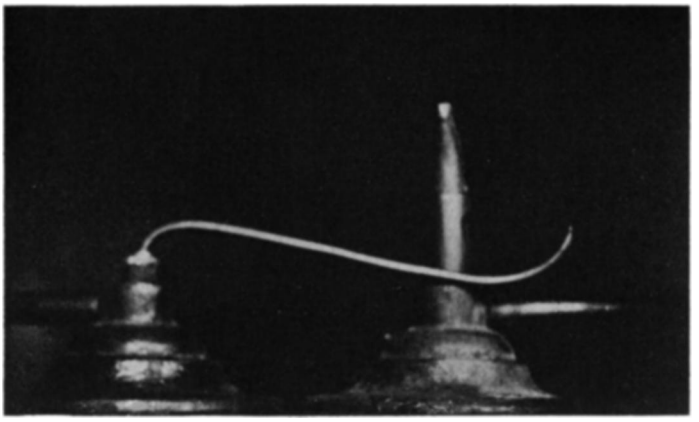

Fig. 5. The two types of Bunsen burners.

at a comfortable distance from the end of the pipet which is in the flame. The thumb forceps are held by the thumb, index, and second fingers of the right hand. As soon as the portion in the flame becomes soft enough to bend of its own weight, the end of the molten portion is grasped firmly with the thumb forceps, and at the same time the glass tubing is removed from the flame, and a lateral pull made from left to right, and continued until the desired caliber of pipet is drawn. The hands are then held in their relative positions for half a minute to allow the glass to cool. We now have, approximately, a straight pipet instead of a crooked one. The excess of glass of the capillary pipet over 85 mm. is broken off.

The very fine capillary pipets are harder to make than the coarse ones. The shank of the coarse capillary pipet is grasped with the thumb and fingers of the left hand while the free end of the pipet is grasped with the thumb forceps in the thumb and fingers of the right hand. The outer portion of the pipet which is near the thumb forceps is held in the fine flame, and the instant the glass becomes soft enough to bend, a lateral pull is made to the right if a moderately coarse pipet is wanted, and to the left if a very fine pipet is wanted.

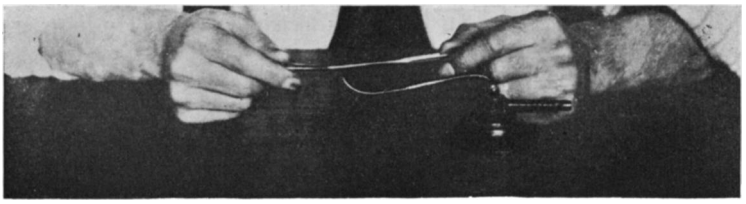

Fig. 6. The position of the hands for making the fine capillary pipet.

Now with the shank of the pipet grasped between the thumb and fingers of the left hand and the free end supported by the point of the forceps in the right hand, the pipet is held in the very fine flame while at the same time gentle pressure upwards is made with the forceps; as soon as one feels the glass giving, the pressure is slightly increased and at the same instant the pipet is removed from the flame.

If on examination of the pipet it is found that the angle is too acute or too obtuse, the defect must be corrected, for if it is not at right angles it can not be successfully used. To correct the angle of the pipet the shank is grasped with the thumb and fingers of the left hand and the point of the forceps is placed on the outer surface; the pipet is then held in the very fine gas flame and gentle upward pressure made with the forceps. As soon as the pipet commences to bend it is immediately removed from the flame and gentle upward pressure made with the forceps. If the angle is too acute, the point of the thumb forceps is placed on the inside of the pipet and it is again placed in the very fine flame, gentle outward pressure being made with the point of the thumb forceps. As soon as the glass commences to bend, the pressure is continued and at the same instant the pipet is removed from the flame. If the angle of the pipet is too obtuse, the point of the thumb forceps is placed on the outside of the fine capillary pipet and it is placed in the fine flame and the angle corrected by bending the pipet inward.

The next step is to break off the excess of the fine capillary pipet over 15 mm. with the points of the thumb forceps, which have been sterilized in the gas flame. The rubber tubing is now fastened to the pipet. The pipet is charged with sterile salt solution or broth by immersing the fine point in the liquor and making suction on the free end of the rubber hose held in the mouth, which draws the desired quantity of fluid into the pipet. After placing the cell on the mechanical stage, the pipet is centered.

*Centering the Point of the Pipet.*—The cell is moved to the right until one half of the field is occupied by the cover slip, and the end of the black line which passes through the longest diameter of the cover slip is in the center of the field. The tube of the microscope is then moved up out of the way and the pipet is placed in the groove of the clamp, 5D, 5d, of the pipet holder, and is pushed inward toward the black line until it is approximately opposite it. The field is then brought into focus, and the point of the pipet is sought by raising and lowering the tube of the microscope. If the pipet is out of the center of the field, it is centered by manipulating the millhead of the carriage which controls the movement in the correct direction. The first pipet having been centered, the second pipet is made by the same technic as was used for making the first. It is adjusted in the same manner also. In addition to

being centered the pipet must be so adjusted that Pipet 1 in moving up or down will in no wise interfere with Pipet 2 and vice versa. Both points should be approximately in the center of the field for this will facilitate the work.

### THE ISOLATION OF LIVING BACTERIA

An area in the emulsion of the pus, rich in bacteria, having been found, the free end of the rubber hose attached to Pipet 1 is placed between the lips and the pipet gradually moved upward with Millhead 2c. As soon as the point of the pipet is in the same focus gentle suction with the mouth draws the desired quantity of the emulsion of pus into the pipet. The pipet is lowered out of focus with Millhead 2c. The mechanical stage is moved to the left of the line which is at right angles to the horizontal line, and to the space below the horizontal line on the under surface of the cover slip. The desired area having been selected the pipet is moved up with Millhead 2c until the point of the pipet is in focus on the under surface of the cover slip; by gentle pressure on the free end of the rubber hose held between the lips, with the mouth, the desired size of droplet is made. The pressure is then relieved and at the same instant the pipet is moved down out of focus by turning Millhead 2c. This process is continued until a series of small droplets are placed on the under surface of the cover slip. Pipet 1 is now moved out of the way and Pipet 2 brought into focus with Millhead 2 C. Small droplets made with Pipet 1 are further diluted with broth from Pipet 2. By this further dilution the bacteria are greatly separated from one another so that isolation becomes easier. This preliminary step is accomplished with a Leitz objective No. 4 and a Huyghenian eye piece No. 5. The isolation of the organism is done with Leitz objective No. 6 or 7 and Huyghenian eye piece No. 2.

The procedure for the isolation of a streptococcus, for example, in pus thus conveyed to the cell is as follows: As soon as a chain is found, the point of the pipet is brought into the same focus by turning Millhead 2C. Then gentle suction is made with the mouth on the free end of the rubber hose attached to the pipet. The instant that the chain of streptococci enters the pipet the suction is stopped and at the same instant the pipet is moved out of focus by turning Millhead 2C, thus preventing all other bacteria from entering the pipet. The mechanical stage is now moved to the left until the line which runs at 95 degrees to the other lines appears. The pipet is then gradually moved up until its point is in focus with one of the small droplets of condensation water which have collected on the under surface of the cover slip.

Gentle pressure with the mouth at once causes the small droplet to increase in size and as soon as the chain of streptococci appears, the pressure is relieved and the pipet instantly moved down out of focus with Millhead 2C. The same technic is followed in the isolation of any other micro-organism present. One soon becomes so expert that the dilution of the original droplet is not necessary, the isolation being made from the first droplet.

In making transplants a method which I find very good is as follows:

The organism having been isolated, Pipet 1 is removed from the pipet holder and a new pipet made and charged with the desired variety of broth and adjusted as previously described. The mechanical stage is now moved to the left, searching for the droplets containing the desired organism, which, when it is found, is drawn into the pipet. The stage is again moved and the organism is dispatched to the under surface of the cover slip, and again picked up with the pipet. The excess of broth is then drawn into the pipet from the droplet. The pipet is now removed from the holder and the broth is carefully drawn to a safe distance from the point of the pipet so that it can be sealed in a gas flame. Then, labeled with the date, hour, and the kind of organism, it is placed in the incubator.

Another method for obtaining cultures of the isolated organisms is that used by Dr. Barber. The organism after isolation is placed in a droplet of culture medium on the under surface of a clean sterile cover slip. The cover slip is then transferred from the cell to a ground-glass concave slide. The preparation of the slide before making the transfer of the cover is as follows: The slide is washed in running hot water and sterilized with alcohol. The ground surface of the slide is then given a liberal coating of vaselin, and in the concave portion of the slide there is placed a small droplet of sterile distilled water. The cover is placed on this slide, the small droplet being on the inside of the concave slide. The slide is then labeled with the time, date, and kind of organism. This method has an advantage over the first method in that one is able to study the growth rate of the organism and at the same time commence an isolated culture. The technic for the study of the growth rate of the colon bacillus, which can be used for the study of other organisms also, has been worked out by Dr. Barber.[1]

The use of the new model of double pipet holder is in nowise confined to the bacteriologist; it can be used by cytologist, embryologist, zoologist, and chemist. I believe that with the double pipet holder many problems can be worked out with greater precision.

[1] Jour. Infect. Dis., 1908, 5, p. 379.